A laboratory study to determine the caries lesion remineralizing potential of novel fluoride- and calcium-containing toothpastes

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Abstract

Background. We conducted a laboratory study to determine the caries lesion remineralization and fluoridation potential of novel fluoride- and calcium-containing toothpastes.

Methods. We created early caries lesions in bovine enamel specimens and assigned them to seven treatment groups based on their surface Vickers microhardness: Clinpro Tooth Crème, CTx4 Gel 1100, Enamelon Fluoride Toothpaste, MI Paste ONE, Crest Cavity Protection, and two fluoride-dose controls (low-F, high-F). We pH cycled the specimens for 10 days using an established model, determined changes in surface microhardness, calculated percent surface microhardness recovery (%SMHr; primary outcome variable), and measured enamel fluoride uptake (EFU). We used a one-way ANOVA for data analysis.

Results. We demonstrated a fluoride-dose response for both %SMHr (low-F control [mean: 9.8; confidence interval: 5.7-13.8], Crest [26.2; 21.8-30.6], high-F control [33.5; 29.4-37.5]) and EFU (low-F control [47; 12-83], Crest [225; 189-260], high-F control [307; 271-342]; all μg F/cm^3). For %SMHr, Clinpro (26.5; 22.5-30.6) and CTx4 (27.3; 23.1-31.5) were similar to Crest, all being superior to Enamelon (15.6; 11.6-19.7), which was superior to MI-ONE (4.3; 0.3-8.3). For EFU, there were no differences between Clinpro (189; 153-224), CTx4 (177; 142-213), Enamelon (196; 161-232), and Crest, all being superior to MI-ONE (66; 30-102).

Conclusions. Our study failed to demonstrate superior remineralizing efficacy of any of the novel toothpastes in comparison to a calcium-free fluoride toothpaste, with two of the four novel toothpastes being inferior. Clinical testing will be required to establish conclusive evidence.

Practical Implications. Clinicians should be aware of the remineralizing potential of new anticaries products.

Key Words. Caries lesion; remineralization; fluoride; calcium; laboratory testing; dentifrices; toothpastes

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INTRODUCTION

Very recently, several fluoride- and calcium-containing toothpastes, claimed to enhance caries lesion remineralization, have become commercially available. These products are over-the-counter fluoride toothpastes with the difference that they are not distributed via conventional paths, such as grocery and drug stores. Instead, they are available exclusively via dental professionals and/or directly from the manufacturer. These toothpastes contain agents such as functionalized β-tricalcium phosphate (*f*TCP),[1] casein phosphopeptide-stabilized amorphous calcium phosphate nanocomplexes (CPP-ACP),[2] nano-hydroxyapatite or amorphous calcium phosphate.[3] Little evidence exists as to their ability to enhance remineralization in the presence of fluoride. Furthermore, an expert panel convened by the American Dental Association Council on Scientific Affairs and the Center for Evidence-Based Dentistry recently advised against the use of 10% CPP-ACP (in the absence of fluoride),[4] with an earlier, similar review concluding that there was insufficient evidence to recommend the use of calcium phosphates in caries prevention.[5]

Consequently, we conducted the present laboratory study to shed some light on the ability of these novel toothpastes to remineralize and fluoridate early caries lesions. Information gained from our study will inform dental professionals about the predicted remineralization efficacy which will aid in the counseling of their patients and the development of treatment plans.

METHODS

Study design. Our laboratory study compared the caries lesion remineralization and fluoridation ability of four fluoride- and calcium-containing toothpastes in comparison to a calcium-free fluoride toothpaste and two fluoride dose controls. We used a 10-day pH cycling remineralization model to assess surface microhardness change (as percent of surface microhardness recovery, %SMHr), and enamel fluoride uptake (EFU) in artificially induced early caries lesions. Data were analyzed using one-way ANOVA. The primary outcome variable was %SMHr.

Specimen preparation, lesion formation and characterization. We prepared enamel specimens obtained from bovine teeth. We cut the tooth sections into round specimens (4 mm diameter) using a hollow-core diamond drill bit. We then ground and polished the specimens to create planar parallel dentin and enamel surfaces. We ground the dentin side flat using 500 grit

silicon carbide paper, followed by grinding and polishing of the enamel side using 1200 grit silicon carbide paper, followed by 2400 and then 4000 grit silicon carbide paper. As a final polishing step, we used a 1-µm diamond suspension on a polishing cloth. Resulting specimens had a thickness range of 1.9 to 2.1 mm. The enamel surface had a minimum polished surface of 2.5 mm diameter in the center of the enamel surface. We then mounted each specimen onto a one-inch square acrylic block with sticky wax and covered the sides of each specimen with an acid resistant nail polish (Sally Hansen Advanced Hard As Nails Nail Polish, USA) so that only the enamel surface was exposed. We prepared and used 13 specimens per treatment group for this study (n = 13).

We used the surface microhardness (SMH) test to assess the mineral status and changes thereof in the enamel specimens. We measured SMH using a Wilson 2100 Hardness Tester by placing five sound enamel baseline indentations spaced vertically 100 µm apart with a Knoop diamond under a 50-gram load in the center of the specimen as shown elsewhere.[6] We determined SMH by measuring the length of the indentations using Clemex CMT HD version 6.0.011 image analysis software. For enamel specimens to be acceptable for use in the study, the mean of the five baseline indentation lengths had to be between 40 and 46 µm with a standard deviation of less than or equal to 3 µm.

We used a modification of the method described by White[7] to create early caries lesions in the specimens. We immersed the enamel specimens for 18 hours at 37º C under static conditions in 40 ml of a demineralization solution with the following composition: 50.0 mM lactic acid, 50% saturated with respect to hydroxyapatite, 0.2% (w/v) Carbopol 907 (BF Goodrich Co., USA), pH adjusted to 5.0 using KOH.

After in vitro demineralization, we measured SMH of the enamel specimens again by placing five indentations 100 µm to the left of the sound enamel indentations. To qualify for inclusion into the study, the mean (n = 5) indentation lengths of the partially demineralized specimens had to be between 140 and 180 µm with a standard deviation of less than or equal to 11 µm. We then assigned specimens to their test toothpastes ensuring that there were no differences in mean post-demineralization indentation lengths between treatment groups.

Test and control toothpastes. Detail of the five test and two control toothpastes can be found in Table 1. We used all test toothpastes as aqueous slurries prepared at a ratio of one part toothpaste to two parts deionized water, thereby mimicking the dilution occurring during toothbrushing. We included two fluoride dose controls using Crest: for the low-F control, we

used an aqueous slurry of Crest at a ratio of 1:100, resulting in an estimated fluoride concentration of approx. 11 ppm F. For the high-F control, we prepared a slurry of Crest with an aqueous solution containing 1,950 ppm F as sodium fluoride at a ratio of 1:2, thereby mimicking a 5,000 ppm F prescription strength toothpaste. We prepared all slurries immediately prior to treatment.

We based our cost estimation (Table 1) on twice-daily usage with 1.5 g toothpaste per toothbrushing session. The cost of each product was determined at BencoDental (Clinpro, Enamelon, MI-One), CariFree (CTx4) and CVS (Crest) online in October 2018 and does not consider tax, shipping costs, special offers, quantity discounts, coupon codes etc. that may affect the actual cost.

pH cycling phase. We utilized a modified version of an established pH cycling model.[7] The main modification was that instead of four daily toothpaste treatments we only treated twice daily to mimic actual human usage. Briefly, we utilized the following sequence every day for 10 d: a 1-min treatment with the assigned toothpaste, 2 h of remineralization, 4 h of cariogenic challenge, 2 h of remineralization, a 1-min treatment with the assigned toothpaste, followed by remineralization overnight. We used artificial saliva (1.5 mM $CaCl_2.2H_2O$; 0.9 mM KH_2PO_4; 130.0 mM KCl, 20.0 mM HEPES, pH adjusted to 7.0 with KOH.) as the remineralization medium. We used a demineralizing solution (100 mM lactic acid, 4.1 mM $CaCl_2$-$2H_2O$; 8.0 mM KH_2PO_4; 0.2% (w/v) Carbopol 907, pH adjusted to 5.0 with KOH) as the cariogenic challenge. We conducted the experiment at room temperature under static conditions. To prevent carry-over, we rinsed all specimens with deionized water after each slurry treatment and solution change. We renewed the remineralization solution and cariogenic challenge daily. Our pH cycling procedure mimics in vivo caries with alternating periods of de- and remineralization with a twice-daily usage of fluoride toothpaste, albeit its limitations (see Discussion) need to be considered in the interpretation of our findings.

Post-pH cycling lesion characterization. After 10 d of pH cycling, we SMH-tested the specimens again as described above by placing five indentations 200 µm to the right of the baseline indentations. We calculated the extent of re- or further demineralization based on the method of Gelhard et al.[8] as follows:

$$\%SMHr = (D-R) / (D-B) \times 100$$

B = mean indentation length (µm) of sound enamel specimen at baseline

D = mean indentation length (μm) after in vitro demineralization

R = mean indentation length (μm) after pH cycling phase

After completion of the SMH testing, we determined the fluoride content of each enamel specimen using the microbiopsy technique to a depth of 200 μm.[9] We placed two drill holes in the bottom right and left corners of the specimen, avoiding the center area, and determined the diameter of the drill holes. We collected the enamel powder from the drill holes, dissolved (20 μl of $HClO_4$, 40 μl Citrate/EDTA Buffer and 40 μl DI water) and analyzed it for fluoride by comparison to a similarly prepared standard curve. We calculated the fluoride data as μg F/cm^3: (μg F × dilution factor / volume of drilling).

Statistical analysis. We tested the data for normal distribution (Shapiro-Wilk test). We then calculated the variables %SMHr and EFU for each specimen and analyzed the data using a one-way ANOVA with the factor 'toothpaste'. We considered %SMHr the primary outcome variable. Where significant differences were indicated, we used Fisher's least significant difference test to determine differences between treatment groups. Based on previous data, we estimated the within-group standard deviations for %SMHr to be 7.0 and for EFU to be 90. With a sample size of 13 specimens per group, the study had 80% power to detect a difference of 6.8 for %SMHr and 86 μg F/cm^3 in EFU between any two groups, assuming two-sided tests each conducted at a 5% significance level.

RESULTS

There were no statistically significant differences between treatment groups for sound enamel microhardness (P = .90) and lesion baseline microhardness (P = 1.0). Table 2 presents the means, standard deviations and results of the statistical analyses for both %SMH and EFU and all treatment groups.

%SMHr

The model demonstrated a fluoride dose-response for %SMHr as the high-F control provided greater remineralization than Crest (P = .018) and the low-F control (P < .0001), with Crest also affording greater remineralization than the low-F control (P < .0001). In comparison to Crest, Clinpro (P = .915) and CTx4 (P = .724) provided similar extents of remineralization. However, both Enamelon (P = .01) and MI-One (P < .0001) were inferior to Crest. MI-One was only

comparable to the low-F control (P = .06) and provided the numerically least extent of remineralization.

EFU

The EFU data also demonstrated a fluoride dose-response as the high-F control provided the greatest fluoride uptake, followed by Crest (P = .02) and the low-F control (P < .0001), with Crest being superior to the low-F control (P < .0001). In comparison to Crest, Clinpro (P = .156), CTx4 (P = .063) and Enamelon (P = .262) provided similar lesion fluoridation, with all being superior to MI-One (all P < .0001).

DISCUSSION

We designed the present laboratory study to evaluate the remineralization potential of several novel fluoride toothpastes, which contain a range of calcium compounds to enhance caries lesion remineralization (Table 1). As not every new toothpaste can be evaluated for its ability to prevent dental caries in an in vivo caries clinical trial, a wide array of model systems to determine the predicted efficacy of fluoride toothpastes has been developed over the past decades. These model systems include in situ (intra-oral),[10] in vitro (laboratory)[11] and animal caries models.[12] While none of these models are like-for-like surrogates for the complexity of in vivo caries,[13] in vitro models are generally considered suitable tools to simulate specific aspects of the caries process.[14] We chose a modified 'White model'[7] in the present study because it is one of the most widely used models to study the ability of toothpastes to remineralize early caries lesions. The primary outcome variable was the change in surface microhardness, which we have shown previously to be more sensitive in determining changes in the mineralization status of early caries lesions than the 'gold standard' transverse microradiography.[15]

One of the key requirements for any laboratory caries model is its ability to demonstrate a fluoride dose-response, which we also observed presently (Table 2). While no clinical data for coronal caries prevention exists for the comparison between 1,100 and 5,000 ppm F (as tested presently), there is a plethora of studies comparing placebo and 1,100 ppm F toothpastes, and several studies comparing toothpastes of higher fluoride concentrations.[16,17] These studies have shown that the relative caries preventive effect of toothpastes increases with increasing fluoride concentrations. In vitro caries models have generally been predictable of the clinical performance of fluoride toothpastes of different concentrations. However, these models often

overestimate potential differences due to their comparatively higher sensitivity. For example, it has been shown that only fluoride concentrations of 1000 ppm and above afford caries prevention in children and adolescents,[17] whereas pH cycling models have demonstrated caries-preventive effects for concentrations as little as 250 ppm F.[18] Furthermore, in vitro caries models can also be designed to yield a net de- or remineralization outcome. Here, we deliberately chose the latter as the tested fluoride- and calcium-containing toothpastes are claimed to provide enhanced remineralization.

Our results indicate that none of the fluoride- and calcium-containing toothpastes provide additional benefits over a fluoride toothpaste not containing calcium, with two being inferior (Table 2). Among the calcium compounds contained in the test toothpastes, CPP-ACP is the most studied and controversial non-fluoride agent. CPP-ACP has been commercialized and can be found in a 5% sodium fluoride varnish, in a gel for prolonged topical application with or without added fluoride (most studied product), and in the presently evaluated fluoride toothpaste. While earlier reviews have been conflicting,[19,20] the consensus based on the current clinical data is that 10% CPP-ACP (topical gel without fluoride) is not recommended for caries prevention and/or management.[4,5,21,22] Our study was the first to evaluate the recently launched CPP-ACP containing fluoride toothpaste. Its poor performance presently may be the result of inadequate fluoride bioavailability as indicated also by the low EFU (Table 2). While CPP-ACP nanocomplexes have been shown to be stable,[23] their disruption will result in the release of ionic calcium. Calcium can then react with fluoride ions and form largely insoluble calcium fluoride-like compounds before reaching the tooth surface or other intra-oral binding sites. This not only applies to CPP-ACP but also to other calcium compounds formulated with fluoride in a single, aqueous base.

We found both Clinpro and CTx4 to be similar to a calcium-free fluoride toothpaste in their ability to remineralize early caries lesions. Clinpro contains *f*TCP,[1] which is also available in a 5% sodium fluoride varnish.[24] A presumably similar toothpaste containing this agent was found to be equivalent to a fluoride toothpaste in an in situ remineralizing study,[25] thereby mirroring present observations (their results on CPP-ACP, however, did not). A comparable in situ caries study was able to demonstrate some synergy between sodium fluoride and *f*TCP in remineralizing early caries lesions, which we did not observe presently.[26] Inherent model differences can be attributed to this discrepancy (e.g. remineralization and demineralization media, sequence of treatments, type and severity of the baseline caries lesion). Unlike Clinpro, CTx4 contains a range of compounds in addition to sodium fluoride (Table 1). Some clinical

data exists on the efficacy of xylitol to prevent caries when co-delivered with sodium fluoride in a toothpaste,[27] while a recent review considered sodium bicarbonate a promising plaque-buffering agent.[28] Some anecdotal clinical evidence suggests that a fluoride-free (nano-)hydroxyapatite containing toothpaste exhibits anti-caries benefits.[29] However, there are no credible studies on its potential in the presence in or comparison to fluoride.

Enamelon, the only test toothpaste containing stannous instead of sodium fluoride, utilizes the 'amorphous calcium phosphate' technology by the earlier and now unavailable Enamelon toothpaste. In contrast to the present day's offering, it contained sodium fluoride and utilized a dual-chamber approach – sodium fluoride and phosphate in one, calcium sulfate in the other, with both mixed during brushing.[30] A similar dual-chamber approach has been successfully tested previously, as two caries clinical trials were able to demonstrate significant caries reduction for a sodium fluoride/dicalcium phosphate dihydrate dual-chamber toothpaste compared to a sodium fluoride only toothpaste.[31,32] Neither the original Enamelon nor the in vivo tested toothpaste are commercially available anymore. Furthermore, we were unable to retrieve any data on the present Enamelon toothpaste. Nonetheless, the sequential[33] or co-delivery[34] of calcium and fluoride has attracted considerable attention by researchers in the past. Results of short-term in vivo studies highlighted that calcium can greatly enhance intra-oral fluoride retention and in particular in dental plaque.[34] This is not surprising given that plaque fluoride concentrations are strongly dependent on plaque calcium concentrations.[35] However, despite more than 20 years of research on this topic, commercialization of a dual- or co-delivery product of calcium and fluoride has been challenging.

While we were primarily concerned with determining the predicted remineralization efficacy of the test toothpastes, we also wanted to highlight the potential cost associated with their every-day use (Table 1). There is an approximate four-fold difference in cost between the fluoride- and calcium-containing toothpastes, with all being substantially more expensive than a calcium-free fluoride toothpaste.

There are limitations that need to be considered when interpreting our findings. As pointed out earlier, no model can replace the complexity of in vivo caries. Here, we investigated the caries lesion remineralization potential and results may have been different if we would have chosen a laboratory model with a net demineralization outcome, such as that developed by Featherstone et al.[18] Irrespective of the model, the subtle pH fluctuations occurring at the dental plaque enamel interface or the gradual release of fluoride and other agents from the toothpaste during brushing, which depend on the formulation's inherent properties, cannot be mimicked

sufficiently in vitro. Furthermore, the inclusion of a dental plaque surrogate (in vitro biofilm) may also be advantageous in future research as fluoride retention is affected by calcium.[35] Lastly, we should not forget that patient compliance, and in particular oral care habits, such as brushing frequency[36] and time,[37] is key to caries prevention afforded by fluoride toothpaste. Paying a premium price for an oral care product may potentially be a motivator to adhere more strictly to professional advice and instructions.

CONCLUSIONS

None of the tested fluoride- and calcium-containing toothpastes provided enhanced caries lesion remineralization or fluoridation in comparison to a fluoride toothpaste, which does not contain calcium. However, further clinical research will be needed to provide comprehensive recommendations as to their usefulness in everyday at-home caries prevention.

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TABLE 1

Test and control toothpastes						
Test toothpastes						
NAME	SHORT NAME	MANUFACTURER	FLUORIDE COMPOUND AND CONCENTRATION	OTHER NOTEWORTHY INGREDIENTS	LOT/BATCH NO.	ANNUAL COST ESTIMATION[1]
Clinpro Tooth Crème	Clinpro	3M ESPE, St. Paul, MN	Sodium fluoride, 0.21% [950 ppm F]	Tri-calcium phosphate	60151	$99
CTx4 Gel 1100	CTx4	Oral Biotech, Albany, OR	Sodium fluoride, 0.24% [1,086 ppm F]	(nano-)Hydroxyapatite, sodium bicarbonate, xylitol	111705	$307
Enamelon Fluoride Toothpaste	Enamelon	Premier Dental, Plymouth Meeting, PA	Stannous fluoride, 0.45% [1,091 ppm F]	Calcium sulfate, monosodium phosphate	PD7341-1	$112
MI Paste ONE	MI-One	GC America Inc., Alsip, IL	Sodium fluoride, 0.24% [1,086 ppm F]	Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP; 10%), xylitol, potassium nitrate (5%)	180117A	$405
Crest Cavity Protection	Crest	Procter & Gamble, Cincinnati, OH	Sodium fluoride, 0.243% [1,099 ppm F]	-	610673	$17

Control toothpastes (fluoride dose controls)						
Crest Cavity Protection[2]	low-F control	Procter & Gamble, Cincinnati, OH	Sodium fluoride, 0.002% [11 ppm F]	-	610673	n/a
Crest Cavity Protection[2]	high-F control	Procter & Gamble, Cincinnati, OH	Sodium fluoride, 1.1% [5,000 ppm F]	-	610673	n/a

[1]See Methods - Test and control toothpastes for cost estimation.

[2]We used Crest Cavity Protection for the preparation of both control toothpaste slurries.

TABLE 2

Means, standard deviations and results of the statistical analyses for all study variables.				
TEST TOOTHPASTES	%SMHr		EFU [µg F/cm^3]	
Clinpro	26.5 ± 8.3	B[1]	189 ± 51	B
CTx4	27.3 ± 6.2	B	177 ± 58	B
Enamelon	15.6 ± 7.7	C	196 ± 72	B
MI-One	4.3 ± 7.5	D	66 ± 34	C
Crest	26.2 ± 7.2	B	225 ± 97	B
CONTROL TOOTHPASTES				
low-F control	9.8 ± 8.6	D	48 ± 31	C
high-F control	33.5 ± 5.2	A	307 ± 81	A

[1]Statistically significant differences between toothpastes within variable are highlighted by different letters.